AREA OF VEIN-ISLETS IN LEAVES OF CERTAIN PLANTS AS AN AGE DETERMINANT

M. R. Ensign

(Received for publication February 26, 1921)

The available evidence in support of a theory of senescence in plants is very meager. The work of Minot (7), Child (2), Hertwig (5), Conklin (3) and others establishes quite definitely that complex animal forms are subject to a gradual retardation of physiological functions; also, that this retardation begins in the embryo and continues with more or less acceleration until death ensues.

Benedict (1) has attempted to show that plants are subject to similar changes of physiological functions. These, he claims, are initiated immediately after fertilization and are registered in the increasing complexity of the vascular ramifications in the leaves of certain dicotyledonous plants. In other words, the relative age of such perennial plants as vines, trees, and shrubs can be detected by determining the relative area of the "tissue islands" or vein-islets formed by the intersecting veins surrounding them. Old or senile plants, therefore, produce leaves whose vein-islets are smaller in area than those in leaves of young plants of the same species grown under similar environmental conditions.

Beginning with these premises, the writer (4) studied the venation of leaves produced by polyembryonic Citrus seedlings (*Citrus grandis*). During the progress of this work certain questions arose regarding the accuracy of the methods employed by Benedict in determining the area of vein-islets in the leaves which he used. In order to shed some light upon this point the work herein reported was undertaken. The data collected are not as extensive as might be desired, but inasmuch as further investigation had to be postponed indefinitely, they are presented for what they may be worth.

Methods and Materials

Leaves from the following plants were studied with reference to their venation: *Berberis vulgaris* L., *Berberis Thunbergii* DC., *Castanea dentata* Borkh., *Quercus alba* L., *Fagus caroliniana* Fernald and Rehder, *Vitis vulpina* L., and an undetermined species of Vitis growing in the physiological greenhouse at Cornell University, Ithaca, New York. The trees and vines grew in the immediate vicinity of Ithaca, and the barberries grew on the university campus.

In collecting the leaves from these plants the trunk diameters were taken as an index of relative age. For comparison two or more plants

growing in the same habitat were selected whose trunk diameters were indicative of youth and of old age respectively. Leaves which had approximately equal light exposure were taken from these plants for study.

Only mature leaves were used, inasmuch as it has been shown by Benedict (1) and by the writer that the area of vein-islets in immature leaves is less than that of vein-islets in mature leaves of the same species.

From five to fifteen leaves from each plant were selected and taken to the laboratory. Portions of each leaf were cleared and stained, and determinations of the size of vein-islets were made according to a previously described method (4). At least four determinations were made from different places[1] on the same leaf. Thus, from each plant from twenty to seventy-five determinations of vein-islet areas were made in order to reduce to a minimum the probable error due to variation.

The method used by Benedict in determining the area of vein-islets is as follows:

> The collected leaves were taken immediately to the laboratory, measured as to length, breadth, and area, and weighed. The venation was then photographed in the following way: a heavy black paper was pasted to a clean glass plate, four by five inches in size. Ten openings, approximately four by ten millimeters in size, were then cut in the black paper. From the same part of each leaf pieces a little larger than the openings were cut, and these were laid over the openings, so that each of the ten leaves was represented. A clear glass plate was then laid over all, and the whole was bound together by elastic bands, placed in the negative holder of an enlarging camera, and photographed at an enlargement of three diameters. Negatives showing the veinlets clearly were obtained after some practice, and from these negatives velox prints were made. . . . The counting (of the number of vein-islets in the opening) was done under a lens, and a sharp needle was used to prick each vein-islet as it was counted on the photograph.

It occurred to the writer that such a method might be conducive to inaccuracy for the following reasons: Leaves growing on the same plant and even on the same twig vary greatly in shape and size. There is no good reason, therefore, for expecting to find such characters as leaf thickness and chlorophyll content constant. In fact, the most casual observation soon discloses the fallacy of such a premise. This being the case, the proportion of vascular bundles visible in the uncleared leaves would vary directly with the leaf thickness and the chlorophyll content.

Only one determination from each leaf appears to be entirely inadequate to overcome the probable error. It would also appear that the inaccuracy would be exaggerated by each step in the photographing, developing, and printing processes, especially when the magnification used was only three diameters.

Experimental Data

In order to find what percentage of the vascular tissue is hidden by chlorophyll, leaves growing under as nearly identical environmental con-

[1] Benedict (1) and the writer (4) have shown that the sizes of vein-islets are quite constant in various places in a single leaf.

ditions as possible were selected. Mature leaves from an unidentified species of grape (*Vitis* sp.) growing in the plant physiological greenhouse at Cornell furnished the necessary material. Only mature leaves were used, since it has been shown that the area of the vein-islets in immature leaves is much less than that in mature leaves.

Portions of each of thirty-eight leaves from a single plant, having the same light exposure, were examined under the projection apparatus, as described in a previous article (4). The magnification used was thirty-eight diameters. A similar portion of each leaf was cleared and stained and determinations of the vein-islet area were made with the same apparatus and at the same magnification. Figure 3, Plate XXIII, shows the vascular tissue of the cleared and stained portion of this grape leaf. Table 1 shows

TABLE 1. *Relative Size of Vein-islets of Uncleared and Cleared Portions of the Same Leaf (Vitis sp.)*

Number of Leaf	Uncleared Portion		Cleared Portion		Vein-islets Hidden in Uncleared Leaves (percent)
	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)	
1	14	.2860	30	.1333	54
2	14	.2860	34	.1176	59
3	21	.1904	33	.1212	37
4	13	.3200	34	.1176	62
5	18	.2222	35	.1143	49
6	25	.1600	30	.1333	17
7	17	.2353	33	.1212	48
8	21	.1904	35	.1143	40
9	15	.2666	30	.1333	50
10	12	.3200	29	.1379	59
11	16	.2500	29	.1379	39
12	17	.2353	31	.1290	46
Average	16.9	.2468	32	.1259	47.5
Mean Average 38 Leaves	16.9±.135	.2485	32±.263	.1240	47.5%

a summary of the data obtained from this study. It is evident from this study that many vein-islets are invisible in the uncleared leaves even with a magnification more than twelve times as great as that used by Benedict. Indeed, the average shows that nearly half the vascular tissue is hidden by the chlorophyll, and in some leaves as much as sixty-two percent is invisible. On the other hand, some leaves show the major portion of their vascular tissue in the uncleared, unstained condition.

Benedict (1) presents data which show that the vein-islets in uncleared leaves grown in the shade are smaller than those in leaves exposed to direct sunlight. These data interpreted in the light of the experimental results shown in table 1 mean, no doubt, that the leaves grown in the direct

light were thicker and contained a larger amount of chlorophyll, so that fewer veins were visible in a unit area. Schuster (8) records a similar condition in the leaves of *Ampelopsis Veitchii.*

Inasmuch as Benedict found a direct correlation between age differences and vein-islet areas in leaves from various perennial plants, some of these plants were studied by clearing and staining. The leaves of *Fagus caroliniana* were taken from plants growing in close proximity to each other yet having large differences in trunk diameters. A summary of the data from this study is presented in tables 2 and 3. Although Benedict did not study the leaves of the beech, he intimates that the leaves of all the woody perennial plants show senescence by the constantly increasing amount of vascular tissue in their leaves as they increase in age. The data in tables 2 and 3 do not show distinctive differences.

TABLE 2. *Relative Size of Vein-islets of Leaves of Fagus caroliniana Fernald and Rehder, from Trees of Different Ages*

Number of Leaf	Trunk Diameter 5.4 cm.		Trunk Diameter 22 cm.	
	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (4 sq. mm.)	Area Vein-islets (sq. mm.)
1	63	.0635	69	.0580
2	62	.0645	64	.0625
3	66	.0606	60	.0666
4	63	.0635	61	.0655
5	64	.0625	65	.0615
6	63	.0635	62	.0645
7	61	.0655	62	.0655
8	63	.0635	63	.0635
9	60	.0666	—	—
10	64	.0625	—	—
Average	62.9	.0637	63.2	.0635

TABLE 3. *Comparison of Size of Vein-islets in Relation to Age of Tree in Fagus caroliniana*

Diameter of Trunk (cm.)	6.2	7.5	18	20
Area of Vein-islets (10 Leaves) (sq. mm.)	.0678	.0640	.0641	.0625

In leaves from specimens of *Castanea dentata* having a trunk-diameter difference of 49.5 cm., Benedict records a difference in vein-islet area of 0.3 square millimeter. In table 4 a summary of the findings in cleared and stained leaves is shown. Here there is but the very slightest difference shown in the area of the vein-islets. The variation found in individual leaves from the same tree shows as great differences.

TABLE 4. *Relation of Size of Vein-islets to Age in Castanea dentata Borkh.*

Diameter of Trunk (cm.)	5.2	3.7	50.4	38
Average Area of Vein-islets of 15 Leaves (sq. mm.)	.0897	.0697	.0876	.0677

A number of determinations made from the cleared leaves of white oak (*Quercus alba*) and of *Platanus occidentalis* revealed no correlation between the size of vein-islets and their relative ages.

The barberry leaves studied were from plants of known age. The department of landscape gardening[2] at Cornell University had several hundred one-year-old seedlings in cultivation. In several places on the campus, barberry plants were known to have been growing from six to twelve years, and were probably several years old when first planted. Leaves from these plants of different ages were cleared, stained, and studied as to venation. A summary of these determinations is presented in tables 5 and 6.

TABLE 5. *Relation of Size of Vein-islets to Age in Berberis vulgaris L.*

Known Age of Plants	1 year	6 years +
Average Area of Vein-islets of 35 Leaves (sq. mm.)...........	.2405	.2378

TABLE 6. *Relation of Size of Vein-islets to Age in Berberis Thunbergii DC.*

Known Age of Plants	2 years	12 years +
Average Area of Vein-islets of 18 Leaves (sq. mm.)...........	.2163	.2196

The conclusions that may be drawn from these results are subject to two interpretations. (1) The age differences may not be sufficiently great to influence very materially the size of the vein-islets. Yet Benedict records instances in which individuals of *Vitis vulpina* with an age difference of not more than three to five years show a positive correlation. (2) It may be that this particular perennial does not register its relative age in its more or less complex nervature. Figures 1 and 2, Plate XXIII, show the nature of venation of *Berberis vulgaris.*

The work up to this point was done while at Cornell in 1916–17. The data presented below were obtained from leaves of *Vitis vulpina* which were gathered from various places near Ithaca, New York. They were preserved in 85 percent alcohol in test tubes until December, 1918, and were in very good condition. Because of the fact, however, that most of the chlorophyll had been extracted, a comparison between the sizes of vein-islets in cleared and uncleared material was not possible.

The plants from which these leaves were taken were selected and marked in the same manner as that described by Benedict (1). The greatest care was taken to secure leaves for comparison that were growing under as nearly similar environmental conditions as possible. Data from cleared and stained leaves only are given in tables 7–12. The results show quite wide variations as to size of vein-islets in the leaves from different vines. The significant fact to be noted, however, is that there is no definite correlation

[2] Courtesy of Professor Hunn.

TABLE 7. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 1 and 2*

Number of Leaves	Vine 0.8 cm. Diameter (5 Annual Rings)		Vine 5.2 cm. Diameter (17 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
12	17.4	.1421	16.6	.1389

TABLE 8. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 3 and 4*

Number of Leaf	Vine 1.3 cm. Diameter (6 Annual Rings)		Vine 10 cm. Diameter (25 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. cm.)	Area Vein-islets (sq. cm.)	No. Vein-islets in Unit Area (2.25 sq. cm.)	Area Vein-islets (sq. cm.)
1	19	.1184	20	.1125
2	17	.1323	21	.1071
3	18	.1250	20	.1125
4	19	.1184	18	.1250
5	18	.1250	17	.1323
6	17	.1323	19	.1184
7	20	.1125	16	.1531
8	19	.1184	17	.1323
9	20	.1125	16	.1531
10	21	.1071	20	.1125
11	20	.1125	17	.1323
12	17	.1323	18	.1250
Average	18.7	.1205	18.2	.1270

TABLE 9. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 5 and 6*

Number of Leaves	Vine 1.3 cm. Diameter (6 Annual Rings)		Vine 5.2 cm. Diameter (17 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
10	13	.1740	16.6	.1389

TABLE 10. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 7 and 12*

Number of Leaves	Vine 3 cm. Diameter (12 Annual Rings)		Vine 6.4 cm. Diameter (18 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
10	21.8	.1054	18.9	.1192

TABLE 11. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 9 and 10*

Number of Leaves	Vine 1 cm. Diameter (5 Annual Rings)		Vine 4 cm. Diameter (15 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
12.............	21.8	.1042	25.2	.0898

TABLE 12. *Relation of Size of Vein-islets to Age in Leaves of Vitis vulpina L., Vines 11 and 12*

Number of Leaves	Vine 1 cm. Diameter (5 Annual Rings)		Vine 6.4 cm. Diameter (18 Annual Rings)	
	No. Vein-islets in Unit Area (2.25 sq. cm.)	Area Vein-islets (sq. mm.)	No. Vein-islets in Unit Area (2.25 sq. mm.)	Area Vein-islets (sq. mm.)
12.............	17	.1342	18.9	.1192

between vein-islet area and age differences. The greatest variation was found in the leaves from vines 9 and 10 (table 10), where the average area of vein-islets showed a difference of 0.0144 sq. mm. The age difference here was ten years according to the number of annual rings. In contrast to these figures, the data in table 8 are interesting. In this case there is an age difference of nineteen years but a difference of but 0.0065 sq. mm. in the average area of vein-islets, the smaller islets being found in the younger plant. For a similar age difference Benedict records a difference in vein-islet area of 0.2553 sq. mm.

It is interesting to make some other comparisons between the sizes of vein-islets as found by Benedict and those obtained by the writer from leaves of the same species, *Vitis vulpina* (table 13). Of course this com-

TABLE 13. *Comparison of Vein-islet Areas Obtained from Individuals of Varying Age but of the Same Species (Vitis vulpina L.)*

Benedict		The Writer	
Number Annual Rings	Vein-islet Area (sq. mm.)	Number Annual Rings	Vein-islet Area (sq. mm.)
5................	0.4845	5	0.1421
6................	0.3983	6	0.1740
6................	0.3684	6	0.1205
6................	0.3983	5	0.1042
11................	0.3690	12	0.1054
16................	0.2966	15	0.0898
17................	0.3310	17	0.1389
16................	0.2966	17	0.1389
17................	0.3160	18	0.1192
25................	0.2503	25	0.1270
Average...........	0.3509		0.1260

parison is not so instructive as one made from cleared and uncleared leaf portions taken from paired plants and studied by the same methods. However, these data compare favorably with the results obtained from the study of the cleared and uncleared leaves of the undetermined species of Vitis shown in table 1. It will be noted that in both cases the uncleared leaves show vein-islet areas from two to three times larger than those from cleared and stained leaf portions.

Discussion

The above data show that any study of leaf venation made from uncleared leaves is wholly unreliable. The varying thickness and chlorophyll content of leaves render many of the smaller veins entirely invisible. Furthermore, some unpublished results obtained by Heinicke[3] do not corroborate the preliminary venation studies of uncleared apple leaves made by Benedict. Heinicke finds no correlation between vein-islet area and the age of a large number of apple varieties. These are of known age, *i.e.*, it is known when they originated as seedlings.

The results herein presented do not show a single instance in which the leaf venation might be taken as an index of the relative ages of the plants in question. While working with yearling Citrus seedlings, a number of grape-fruit leaves were obtained from some of the oldest trees in the vicinity of Miami, Florida, and it was found that the venation of the leaves from the yearling plants was identical with that from the older trees. A similar condition was found in regard to the venation of some orange leaves taken from a plant growing in the Sage greenhouse at Cornell. This plant was probably ten or fifteen years of age. These similarities in venation seem to be indicative of something more than mere coincidences.

As intimated at the beginning, it is highly desirable that more data be secured bearing upon this problem. There are certain phases which require more elucidation before satisfactory conclusions can be derived. It may be that the venation of the uncleared leaves of *Vitis vulpina* and other plants with which Benedict worked shows some correlation with age which the cleared leaves fail to reveal. Such a possibility, however, does not seem tenable.

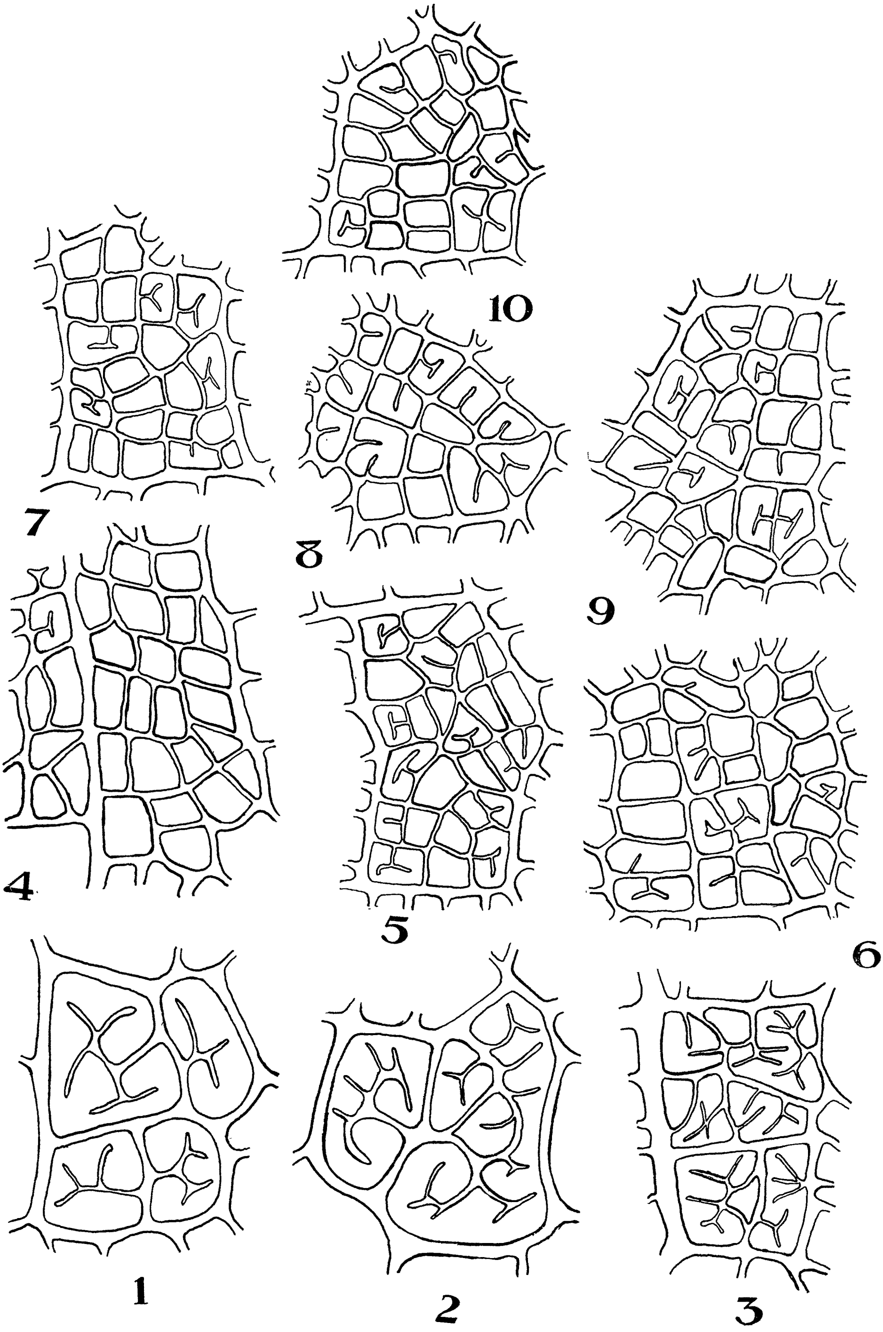
Just as this goes to press the following statement comes from August Henry, Royal Society of Dublin:

> I tried this [venation vs. age] in the various species and hybrids while working on my paper on "The Origin of the London Kane." In this I dealt with the genus Platanus (*Proc. Royal Irish Acad.*) without any very conclusive results. Here the question lay in regard to whether trees produced of cuttings were as old as the original, or only as young as the time the cuttings were started.

Summary and Conclusions

1. From seventeen to sixty-two percent of the vein-islets are invisible in uncleared leaves of *Vitis* sp.

[3] A. J. Heinicke, assistant professor of horticulture, Cornell University.



ENSIGN: VEIN-ISLET AREA IN LEAVES.

2. No correlation was evident between the age of the following plants as indicated by their trunk diameters and the vein-islet area of their leaves: *Fagus caroliniana* Fernald and Rehder; *Castanea dentata* Borkh.; *Berberis vulgaris* L.; *B. Thunbergii* DC., and *Vitis vulpina* L.

BIBLIOGRAPHY

1. **Benedict, H. M.** Senile changes in leaves of *Vitis vulpina* L. and certain other plants. New York Agr. Exp. Sta. (Cornell) Memoir **7**: 281–370. 1915.
2. **Child, C. M.** The process of reproduction in organisms. Biol. Bull. **23**: 1–39. 1912.
   Senescence and rejuvenescence. pp. 481. Chicago, 1915.
3. **Conklin, E. G.** Cell size and nuclear size. Jour. Exp. Zool. **12**: 1–98. 1912.
   The size of organisms and their constituent parts in relation to longevity, senescence and rejuvenescence. Pop. Sci. Mo. **83**: 178–198. 1913.
4. **Ensign, M. R.** Venation and senescence of polyembryonic Citrus plants. Amer. Jour. Bot. **6**: 311–329. 1919.
5. **Hertwig, R.** Ueber Korrelation von Zell- und Kerngrösse und ihre Bedeutung für die geschlechtliche Differenzirung und die Teilung der Zelle. Biol. Centralb. **23**: 194–223. 1903.
6. **Jennings, H. S.** Age, death and conjugation in the light of the work on lower organisms. Pop. Sci. Mo. **80**: 563–577. 1912.
   Effect of conjugation in Paramecium. Jour. Exp. Zool. **14**: 279–391. 1913.
7. **Minot, C. S.** The problem of age, growth and death. pp. 280. New York, 1908.
8. **Schuster, W.** Die Blattaderung des Dicotylenblattes und ihre Abhängigkeit von äusseren Einflussen. Ber. Deutsch. Bot. Ges. **26**: 194–237. 1908.

EXPLANATION OF PLATE XXIII

All figures are drawings made from the projection of the cleared and stained leaves. × 38.

FIG. 1. Venation of a leaf from 6-year-old barberry (*Berberis vulgaris*).

FIG. 2. Venation of a leaf from 1-year-old barberry (*Berberis vulgaris*).

FIG. 3. Venation of a mature leaf from undetermined species of Vitis.

FIG. 4. Venation of grape leaf (*Vitis vulpina*) having a trunk diameter of 1.3 cm. and showing 6 annual rings.

FIG. 5. Venation of a leaf from chestnut (*Castanea dentata*) having a trunk diameter of 38 cm.

FIG. 6. Venation of a leaf of grape (*Vitis vulpina*) having a trunk diameter of 10 cm. and showing 25 annual rings.

FIG. 7. Venation of a leaf from grape (*Vitis vulpina*) having a trunk diameter of 0.8 cm. and showing 5 annual rings.

FIG. 8. Venation of a leaf from grape (*Vitis vulpina*) having a trunk diameter of 5.2 cm. and showing 17 annual rings.

FIG. 9. Venation of a leaf from grape (*Vitis vulpina*) having a trunk diameter of 6.4 cm. and showing 18 annual rings.

FIG. 10. Venation of a leaf from chestnut (*Castanea dentata*) having a trunk diameter of 3.7 cm.